Properties of Caseins Modified by Treatment with Carboxypeptidase A

The two major components of cow's milk casein, α_{s1}- and β-casein, have hydrophobic amino acid sequences in the C-terminal portion of the polypeptide chain. α_{s1}-Casein terminates in -Leu-Leu-Try (2), whereas β-casein most probably terminates in -Ileu-Ileu-Val (3). This is the case with all of the genetic variants of these proteins so far examined. The major whey proteins also contain hydrophobic amino acids at the C-terminus; β-lactoglobulin (1) ends in -Ileu, whereas α-lactalbumin (7) terminates in -Leu. With the whey proteins, the C-terminal amino acid can be removed by the action of carboxypeptidase A, leaving a still crystallizable (1, 7) residual protein. The effect of removal of C-terminal amino acids from the caseins by the action of carboxypeptidase A has now been studied with α_{s1}- and β-casein to determine possible alterations of properties such as aggregation, stabilization by κ-casein, and electrophoretic mobility.

The genetic variants, α_{s1}-A and β-casein C, chosen for this study were prepared by the method of Thompson (5). Five hundred milligrams of each variant was dissolved at pH 8.2 in a volume of 50 ml, and 5 mg (100:1 ratio protein to enzyme) of carboxypeptidase A (treated with DFP prior to use) was added as a water suspension of crystalline material. The reactions were carried out in an unbuffered system at 37 C. α_{s1}-A was reacted for 5 hr, by which time one residue of tryptophan and

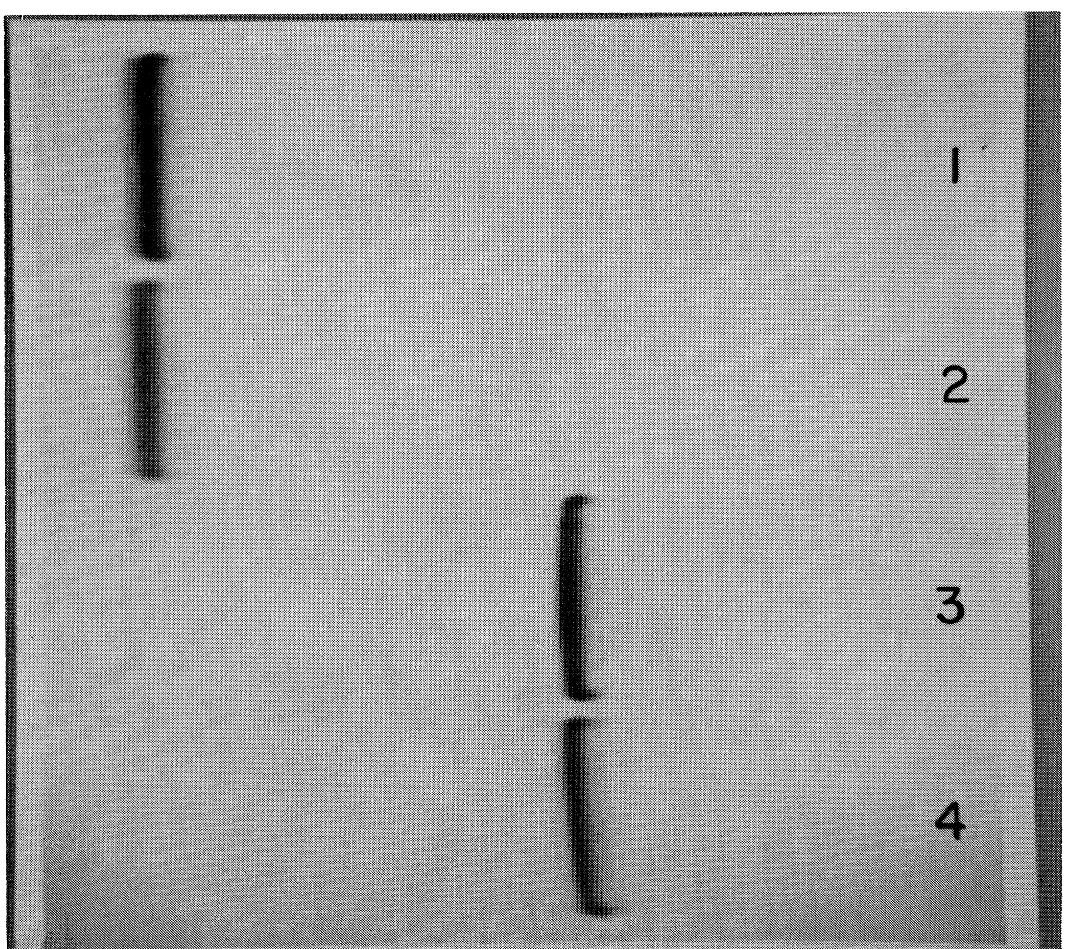

Fig. 1. Polyacrylamide gel electrophoresis, pH 9.1, 4.5 M urea, of (1) α_{s1}-casein A untreated, (2) α_{s1}-casein A treated with carboxypeptidase A, (3) β-casein C untreated, and (4) β-casein C treated with carboxypeptidase A. Solutions applied to the gel contained 5 mg per ml.

at least one residue of leucine were released; β-casein C was reacted for 6 hr, during which one residue of valine and almost two isoleucines were released. A 1.0-ml sample was withdrawn after the above reaction times for amino acid analysis. The sample was added to 0.5 ml 20% trichloroacetic acid, centrifuged in the cold, and the decanted supernatant kept at −20 C until analyzed. For physical studies, the reacted protein was obtained by centrifuging off the suspended carboxypeptidase in the cold after the reaction was completed, warming to 20 C, and rapidly acidifying to pH 4.6. After washing the residue at pH 4.6 several times with distilled water, it was dissolved at pH 7.0 and lyophilized without dialysis.

Amino acid analysis of trichloracetic acid supernatant of α_{s1}-casein A following carboxypeptidase reaction showed that one tryptophan and one leucine residue were released, calculated from a molecular weight of 27,500. β-Casein c released one residue of valine and 1.7 residues of isoleucine per 24,000 molecular weight. As would be expected with α_{s1}-casein A, the absorbancy at 280 mμ decreased from 10.1 to 8.5. Figure 1, gel electrophoresis at pH 9.1, illustrates that alteration of the C-terminal sequence of α_{s1}-casein A results in a slight but perceptible increase in the mobility of this variant. This effect is also observed with the B and C variants of this protein. It is doubtful that the removal of one residue of tryptophan has had an appreciable effect on the net negative charge of the molecule at pH 9.1, as has been observed upon removing histidine from β-lactoglobulin (1), but perhaps a more meaningful explanation is that an alteration of the C-terminal sequence has resulted in a conformational change in the molecule which can be differentiated by the molecular sieving effect of polyacrylamide gels. Carboxypeptidase-treated β-casein showed no change in mobility on polyacrylamide gel electrophoresis as compared with the parent protein (Figure 1).

Carboxypeptidase treatment of α_{s1}-casein A did not result in any change in several characteristic properties of this protein. First, it is quantitatively precipitated by dilute $CaCl_2$ at 37 C and is fully stabilized against precipitation at a weight ratio of 10:1 by κ-casein in the presence of $CaCl_2$. Secondly, no change in sedimentation behavior was noted at pH 7.0, phosphate buffer, in the ultracentrifuge, where pronounced aggregation of this protein is apparent. Clearly, however, β-casein monomer-polymer formation at pH 7.0 (4) is markedly affected by the removal of C-terminal valine and two residues of isoleucine (Figure 2). After equilibration of the control and treated β-caseins at 8.5 C for at least 24 hr, the polymer formation of β-casein after carboxypeptidase A treatment is markedly decreased. To observe

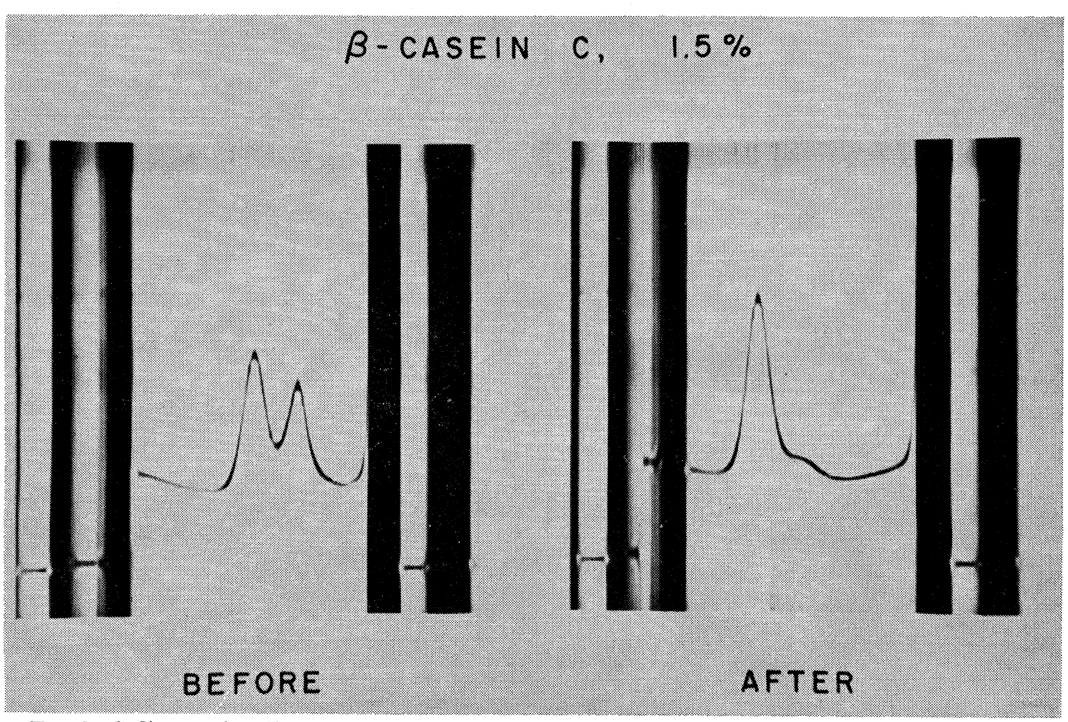

FIG. 2. Sedimentation diagrams of β-casein C before and after treatment with carboxypeptidase A. The centrifuge was operated at 59,780 rpm at 8.5 C. Protein was equilibrated for at least 24 hr prior to sedimentation at 8.5 C in 0.20 M phosphate buffer at pH 7.0.

if the decrease in polymer formation of carboxypeptidase-treated β-casein C was the result of enzymatic degradation during equilibration time, both the untreated and treated β-caseins (1.5% solutions) were incubated at 8.5 C for 24 hr in the pH 7.0 buffer. Following gel electrophoresis of these proteins, no degradation was apparent. Loss of hydrophobic C-terminal amino acids, then, affects the degree of polymer formation at this temperature. It has been proposed that β-casein polymers in solution exist either as more or less stiff rods or as interlinked flexible coils (4), although no one is quite certain of which. However, Waugh (6) indicates a relationship of the extremely high content of apolar side-chains found in β-casein to the strong association of this protein, and our results on the removal of a few apolar side-chains strengthen this suggestion. Of further interest is the influence of higher temperature on the association of treated β-casein; at 20 C, where β-casein is normally fully aggregated, the carboxypeptidase A-treated casein still contains about 25% of the monomer, which indicates a change in the equilibrium constant for the monomer-polymer association.

M. P. THOMPSON
E. B. KALAN
and
RAE GREENBERG
Eastern Regional Research Laboratory[1]
Philadelphia, Pennsylvania

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[1] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.